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plants <i>Lemna gibba</i> PMRA Submission #:{	}	W. W.	EF	A MRID#:45666704
Data Requirement:	PMRA Data Code: EPA DP Barcode: OECD Data Point: EPA MRID: EPA Guideline:	{} D283017 {} 45666704 123-2		ent Number
Common name: Glyfos (Chemical name: IUPAC: CAS nai CAS No	glyphosate as the isopropyliglyphosate as the isopropyliglyphosate as the isopropyliglyphosate as the isopropylight. Not reported in the isopropylight. 1071-83-6 in the isopropylight.		Purity: 31.0% gh	phosate acid
			· •	
Primary Reviewer : Rebec Staff Scientist, Dynamac C	· · · · · · · · · · · · · · · · · · ·	Signature: Date: 10/17/	Rebeccu Bryan	
QC Reviewer: Teri Myers Staff Scientist, Dynamac C		Signature: Date: 10/17/	Dr. Shyn	
Primary Reviewer: Steph {EPA/OECD/PMRA}	en Carey	Date: {	11/08/02 8	
Secondary Reviewer(s):{ {EPA/OECD/PMRA}	}	Date: {	}	
EPA PC Code 103		IRA]		
Date Evaluation Complete	t ed: {dd -mmm-yyyy} , N o	vender 1th	2002	
	- 555557.	· ·		

December 13, 2000, and experimental termination date December 20, 2000. Final report issued December 19, 2001.

Data Evaluation Report on the acute toxicity of Glyfos (glyphosate product) to aquatic vascular plants Lemna gibba

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants Duckweed, Lemna gibba G3, were exposed to Glyfos (glyphosate product) at mean measured concentrations of 0.94, 1.9, 3.9, 7.9, 16, and 30 mg glyfos/L under static conditions. Nominal concentrations were 0.94, 1.9, 3.8, 7.5, 15, and 30 mg glyfos/L. Plant number was significantly reduced at the 16 mg glyfos/L (4.9 mg a.e./L) treatment group; the NOEC for plant number was 7.9 mg glyfos/L (2.4 mg a.e./L) and the EC₅₀ was 25 mg glyfos/L (7.7 mg a.e./L). Frond number was affected at lower concentrations than plant number, with significant reductions occurring at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment group; the NOEC for frond number was 0.94 mg glyfos/L (0.29 mg a.e./L) and the EC₅₀ was 27 mg glyfos/L (8.3 mg a.e./L). Percent inhibition for frond number was 4.7, 9.7, 5.3, 4.1, 29, and 56% in the 0.94, 3.9, 7.9, 16, and 30 mg glyfos/L treatment groups, respectively, compared to the dilution water control. At test termination, the percentage of necrotic and chlorotic fronds was higher in the 16 and 30 mg glyfos/L (2.4 and 4.9 mg a.e./L) treatment groups, compared to the dilution water control.

This toxicity study is scientifically sound and satisfy the guideline requirements for an acute toxicity study with aquatic vascular plants. However, the percent recoveries of the test concentrations were 102-113% at test termination. As a result, this study is classified as CORE for a formulated product.

Results Synopsis

Test Organism: Lemna gibba G3

Test Type: Static

Plant number

 EC_{05} : Not reported 95% C.I.: N/A

NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L)

LOEC: 16 mg glyfos/L (4.9 mg a.i/L)

Probit Slope: $4.69 \pm 0.660 \ (4.76 \pm 0.669)$

 EC_{50}/IC_{50} : 25 mg glyfos/L (7.7 mg a.e./L)

95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number

 EC_{05} :

Not reported

95% C.I.: N/A

NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L) LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)

Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)

EC₅₀/IC₅₀: 27 mg glyfos/L (8.3 mg a.e./L) 95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L) Data Evaluation Report on the acute toxicity of Glyfos (glyphosate product) to aquatic vascular plants Lemna gibba

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Subdivision J, § 123-2. The following deviations were observed:

- 1. This study was conducted under static conditions. US EPA recommends that 7-day duckweed tests be conducted under static renewal conditions (1 renewal for a 7-day test).
- 2. The carbon source of the media was not reported.
- 3. The study author failed to report the OECD test chemical physical characteristics (i.e., water solubility, vapor pressure, molecular weight and specific activity).

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and No Data Confidentiality

statements were provided.

A. MATERIALS:

1. Test Material

Glyfos (glyphosate product)

Description:

Yellow liquid

Lot No./Batch No.: 80821-47

Purity:

31.0% glyphosate acid

Stability of Compound

Under Test Conditions: Measured concentrations on day 0 ranged from 95.7 to 104% of nominal concentrations and measured concentrations on day 7 ranged from 102 to 113% of nominal concentrations, showing that the test material was stable under test conditions. OECD requirements were not reported.

Water solubility: Not reported Vapor pressure: Not reported Specific activity: Not reported Molecular weight: Not reported

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored at ambient room temperature.

2. Test organism:

Name: Duckweed, Lemna gibba EPA requires a vascular species: Lemna gibba.

Strain, if provided: G3

Source: Cultures maintained at Wildlife International, Ltd., Easton, Maryland

Age of inoculum: ≥14 days

Method of cultivation: M-Hoagland's Medium

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B. STUDY DESIGN:

- a) Range-finding Study: Not reported; the authors indicate that the definitive study was based on the results of an exploratory range-finding test. No further information is provided.
- b) Definitive Study

Table 1. Experimental Parameters

	D . 11	Remarks
Parameter	Details	Criteria
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	≥14 days Same as test. Prior to the test the plants were actively growing and healthy.	
Test system static/static renewal/ renewal rate for static renewal:	Static	EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).
Incubation facility	Environmental chamber	
Duration of the test	7 days	EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL 100 mL	
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	M-Hoagland's medium without EDTA or sucrose 4.6 5.3-5.6 None Not reported	EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP. Chelators are not recommended.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	

		Remarks	
Parameter	Details		
		Criteria	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Wildlife International, Ltd. purified well water 5.0 ± 0.1 Not reported Not reported Not reported Below levels of concern (Appendix 2, pp. 28-29) Not detected Not reported	EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.	
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution		
Aeration or agitation	Not reported		
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable		
Number of replicates control: solvent control: treatments:	3 N/A 3		
Number of plants/replicate	5 plants per replicate	EPA requires 5 plants.	
Number of fronds/plant	3 fronds per plant	EPA requires 3 fronds per plant.	
Test concentrations nominal:	0.94, 1.9, 3.8, 7.5, 15, and 30 mg glyfos/L 0.94, 1.9, 3.9, 7.9, 16, and 30	EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.	
	mg glyfos/L (0.29, 0.58, 1.2, 2.4, 4.9, and 9.1 mg a.e./L).	221 01 321 p1 0g1 custore.	
Solvent (type, percentage, if used)	None		

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Parameter	Details	Remarks Criteria
Method and interval of analytical verification	HPLC; 0 and 7	
Test conditions temperature: photoperiod: light intensity and quality:	24.3-25.2°C Continuous 4750 ± 464 lux (range: 4360- 5500 lux), warm-white fluorescent lighting.	EPA temperature: 25°C EPA photoperiod: continuous EPA light: 5.0 Klux (±15%)
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds and toxicity symptoms	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	0, 3, 5, and 7 days.	
Other observations, if any	Colony break-up and root destruction	
Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Mean and replicate data provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Mean frond number decreased as test concentrations increased, when compared to the dilution water control. Percent inhibition was 4.7, 9.7, 5.3, 4.1, 29, and 56% in the 0.94, 3.9, 7.9, 16, and 30 mg glyfos/L treatment groups, respectively,

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compared to the dilution water control. The mean frond numbers of the 16 and 30 mg glyfos/L treatment groups were significantly reduced when compared to the dilution water control. The significant reduction of the 1.9 mg glyfos/L treatment group was not considered treatment-related.

At test termination, the percentage of necrotic and chlorotic fronds were higher in the 16 and 30 mg glyfos/L treatment groups, compared to the dilution water control.

Table 3: Effect of Glyfos (glyphosate product) on plant and frond number of Duckweed, Lemna gibba

Treatment ¹ (measured	Initial plant number/	Observ	ations	Initial frond number/test	Observa	Observations	
and nominal concentration (mg/L)	test solution	Mean plant number	% inhibition	solution	Mean frond number	% inhibition ^a	
Dilution water control	15	21		45	113.0		
Solvent control	N/A	N/A	N/A	N/A	N/A	N/A	
0.94 (0.94)	15	23	-9.5	45	107.7	4.7	
1.9 (1.9)	15	20	4.8	45	102.0*	9.7	
3.9 (3.8)	15	23	-9.5	45	107.0	5.3	
7.9 (7.5)	15	22	-4.8	45	108.3	4.1	
16 (15)	15	19	9.5	45	79.7*	29	
30 (30)	15	8	62	45	49.7*	56	
Reference chemical (if used)	Not applicable.						

¹ Mean measured concentrations with nominal concentrations reported within parentheses.

^a % inhibition was determined by comparing the treatment groups to the dilution water control.

^{*} Significantly different (p<0.05) from the dilution water control.

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Table 4: Statistical endpoint values.

Statistical Endpoint	frond No.	growth rate	other parameter
NOAEC or EC ₀₅ (mg/L)	7.9	Not applicable	Not applicable
LOAEC (mg/L)	Not reported		
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	27 (21 to 33)		
other (IC ₂₅ /EC ₂₅)	Not reported		
Reference chemical NOAEC IC ₅₀ /EC ₅₀	Not applicable		

B. REPORTED STATISTICS: Frond number on day 7 was analyzed using mean measured concentrations. The Shapiro-Wilk's test and Bartlett's test were used to confirm the normality and homogeneity of variances. ANOVA and Dunnett's test was used to compare treatment groups to dilution water control. The TOXSTAT statistical program to analyze the plant numbers, frond numbers, and the percentages of dead and necrotic fronds. The EC₅₀ and 95% confidence limits were determined through linear interpolation via SAS software; the EC₅₀ and 95% confidence interval for the acid equivalent concentrations were converted from these values using the equation provided in the study.

Frond number

NOEC: 7.9 mg glyfos/L (2.4 mg a.i./L)

LOEC: Not reported

EC₅₀/IC₅₀: 27 mg glyfos/L (8.2 mg a.i./L) 95% C.I.: 21 - 33 mg glyfos/L (6.4 - 10 mg a.i./L)

C. VERIFICATION OF STATISTICAL RESULTS:

The NOEC and LOEC for plant number and frond number were determined using ANOVA, followed by Dunnett's test via TOXSTAT statistical software. Data were determined to be normally distributed and the variances were homogeneous prior to these analyses. The EC₅₀ values for the formulated end-use product and the acid equivalent concentrations were estimated by conducting two separate analyses to compare both sets of these concentrations to the plant number and frond number responses using the probit method via Nuthatch statistical software. In most cases this method of analysis provided slightly different values than simply using the acid equivalent equation in the study to convert the formulated end-use product estimates, because the slope of the dose-response relationship slightly changed.

Plant number

NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L) Probit Slope: $4.69 \pm 0.660 \ (4.76 \pm 0.669)$ LOEC: 16 mg glyfos/L (4.9 mg a.i/L)

 EC_{50}/IC_{50} : 25 mg glyfos/L (7.7 mg a.e./L)

95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number

NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L)

LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)

Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)

EC₅₀/IC₅₀: 27 mg glyfos/L (8.3 mg a.e./L)

95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L)

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D. STUDY DEFICIENCIES:

This study was conducted under static conditions. US EPA recommends that 7-day duckweed tests be conducted under static renewal conditions (1 renewals for a 7-day test). However, the percent recoveries of the test concentrations were 102-113% at test termination.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the EC_{50} for frond number based on the formulated product was identical to the study authors', while the EC_{50} estimate based on the acid equivalent concentrations was slightly different (8.3 vs. 8.2 mg a.e./L). These differences were due to the fact that the reviewer estimated the EC_{50} value and 95% confidence interval for the formulated end-use product concentrations and acid equivalent concentrations separately, while the study authors simply used the equation in the study to convert the formulated product EC_{50} to reflect acid equivalent concentrations. These methods produced a slightly different set of numbers because the slope of the dose-response relationship marginally changed upon re-analysis of the values with the converted acid-equivalent concentrations. In this study, the reviewer also determined lower LOEC and NOEC values for this endpoint. The study authors dismissed the significant reduction in frond number at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment level, because the response for this endpoint was not dose-dependent.

The measured concentrations 0.94, 1.9, 3.9, 7.9, 16, and 30 mg glyfos/L were equivalent to 0.29, 0.58, 1.2, 2.4, 4.9, and 9.1 mg a.e./L of glyphosate acid.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfy the guideline requirements for an acute toxicity study with aquatic vascular plants. However, the percent recoveries of the test concentrations were 102-113% at test termination. As a result, this study is classified as CORE for a formulated product. Frond number was affected at lower concentrations than plant number, with significant reductions occurring at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment group; the NOEC for frond number was 0.94 mg glyfos/L (0.29 mg a.e./L) and the EC₅₀ was 27 mg glyfos/L (8.3 mg a.e./L).

Plant number

NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L)

LOEC: 16 mg glyfos/L (4.9 mg a.i/L)

Probit Slope: $4.69 \pm 0.660 \ (4.76 \pm 0.669)$ EC₅₀/IC₅₀: 25 mg glyfos/L (7.7 mg a.e./L)

95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number

NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L)

LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)

Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)

 EC_{50}/IC_{50} : 27 mg glyfos/L (8.3 mg a.e./L)

95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L)

Endpoint(s) Affected: frond number

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III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.4400: Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II. Washington, DC.

ASTM Standard Guide 1218-90E. 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. American Society for Testing and Materials. Philadelphia, Pennsylvania..

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West, Inc. and Gulley, D.D. 1996. TOXSTAT Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Norberg-King, T. J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. Version 2.0. U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, Minnesota. Technical Report 03-93.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

plant number

File: 6704pn

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	512.286	85.381	44.819
Within (Error)	14	26.667	1.905	
Total	20	538.952		

Critical F value = 2.85 (0.05, 6, 14)

Since F > Critical F REJECT Ho: All groups equal

plant number

File: 6704pn

Transform: NO TRANSFORMATION

	DUNNETTS TEST - TABLE 1 OF 2		Ho:Control <treatment< th=""><th></th></treatment<>		
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1 2 3 4 5 6	neg control 0.94 mg/L 1.9 mg/L 3.9 mg/L 7.9 mg/L 16 mg/L 30 mg/L	21.333 22.667 20.000 23.333 22.333 18.000 8.000	21.333 22.667 20.000 23.333 22.333 18.000 8.000	-1.183 1.183 -1.775 -0.887 2.958 11.831	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

plant number

File: 6704pn

Transform: NO TRANSFORMATION

	DUNNETTS TEST - T	ABLE 2 OF	2 Ho:	Control <t< th=""><th>reatment</th></t<>	reatment
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	0.94 mg/L	3	2.851	13.4	-1.333
. 3	1.9 mg/L	3	2.851	13.4	1.333
4	3.9 mg/L	3	2.851	13.4	-2.000
5	7.9 mg/L	3	2.851	13.4	-1.000
6	16 mg/L	3	2.851	13.4	3.333
7	30 mg/L	3	2.851	13.4	13.333

plant number

File: 6704pn

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	21.333	21.333	22.000
2	0.94 mg/L	3	22.667	22.667	22.000
3	1.9 mg/L	3	20.000	20.000	21.889
4	3.9 mg/L	3	23.333	23.333	21.889
5	7.9 mg/L	3	22.333	22.333	21.889
6 · 7	16 mg/L 30 mg/L	3	18.000 8.000	18.000 8.000	18.000 8.000

plant number

File: 6704pn Transform: NO TRANSFORMATION

WILLIAMS TEST	(Isotonic	regression	model)	TABLE 2 O	F 2
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control 0.94 mg/L 1.9 mg/L 3.9 mg/L 7.9 mg/L 16 mg/L 30 mg/L	22.000 22.000 21.889 21.889 21.889 18.000 8.000	0.592 0.493 0.493 0.493 2.958 11.832	*	1.76 1.85 1.88 1.89 1.90	k= 1, v=14 k= 2, v=14 k= 3, v=14 k= 4, v=14 k= 5, v=14 k= 6, v=14

s = 1.380

Note: df used for table values are approximate when v > 20.

frond number

File: 6704fn

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	9434.000	1572.333	73.213
Within (Error)	14	300.667	21.476	
Total	20	9734.667		

Critical F value = 2.85 (0.05, 6, 14)

Since F > Critical F REJECT Ho: All groups equal

frond number

File: 6704fn

Transform: NO TRANSFORMATION

	DUNNETTS TEST - TABLE 1 OF 2		Ho:Control <treatment< th=""></treatment<>			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG	
1 2	neg control 0.94 mg/L	113.000 107.667	113.000 107.667	1.410		

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3	1.9 mg/L	102.000	102.000	2.907	*
4	3.9 mg/L	107.000	107.000	1.586	
5	7.9 mg/L	108.333	108.333	1.233	
6	16 mg/L	79.667	79.667	8.809	*
7	30 mg/L	49.667	49.667	16.738	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

frond number

File: 6704fn Transform: NO TRANSFORMATION

GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL	
1 neg control 3	
2 0.94 mg/L 3 9.573 8.5 5.333	3
3 1.9 mg/L 3 9.573 8.5 11.000)
4 3.9 mg/L 3 9.573 8.5 6.000)
5 7.9 mg/L 3 9.573 8.5 4.667	1
6 16 mg/L 3 9.573 8.5 33.333	3
7 30 mg/L 3 9.573 8.5 63.333	\$

frond number

Transform: NO TRANSFORMATION File: 6704fn

	WILLIAMS TEST (Isoton	LC	regression model)	TABLE 1 OF	2
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	113.000	113.000	113.000
2	0.94 mg/L	3	107.667	107.667	107.667
3	1.9 mg/L	3	102.000	102.000	105.778
4	3.9 mg/L	3	107.000	107.000	105.778
5	7.9 mg/L	3	108.333	108.333	105.778
6	16 mg/L	3	79.667	79.667	79.667
7	30 mg/L	3	49.667	49.667	49.667

frond number

File: 6704fn Transform: NO TRANSFORMATION

WILLIAMS TEST	(Isotonic	regression	model)	TABLE 2 O	F 2
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control 0.94 mg/L 1.9 mg/L 3.9 mg/L 7.9 mg/L 16 mg/L 30 mg/L	113.000 107.667 105.778 105.778 105.778 79.667 49.667	1.410 1.909 1.909 1.909 8.809 16.738	* * * *	1.76 1.85 1.88 1.89 1.90	k= 1, v=14 k= 2, v=14 k= 3, v=14 k= 4, v=14 k= 5, v=14 k= 6, v=14

s = 4.634

Note: df used for table values are approximate when $\ensuremath{\text{v}}\xspace > 20\ensuremath{\text{.}}$